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(54) Title: USE OF CLIOQUINOL FOR TREATING HELICOBACTER, INCLUDING *H. PYLORI*, INFECTIONS AND RELATED DISEASES

(57) Abstract

A method for treating Helicobacter, including *H. pylori*, infection related disease, including gastritis, chronic gastritis, gastric ulcers, duodenal ulcers, gastric carcinoma and non ulcer dyspepsia characterized by the administration to a human of an effective quantity of clioquinol in an oral vehicle for gastric delivery, and preferably simultaneous administration of an effective quantity of a bismuth salt. Drugs for this method and use of clioquinol to prepare these drugs.

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5 BACKGROUND

10 USE OF CLIOQUINOL FOR TREATING HELICOBACTER, INCLUDING H.PYLORI, INFECTIONS AND RELATED DISEASES.

BACKGROUND

15 *Helicobacter pylori* (formely called *Campylobacter pylori*), a gram-negative, curved, motile, microaerophilic rod, was firstly connected with gastritis in 1982 (Warren and Marshall 1983, Marshall and Warren 1984). It has now become clear that *H. pylori* infection is highly associated, although not sufficient in and of itself, with lesions variably known as chronic 20 superficial, diffuse antral or type B gastritis and peptic ulcer as well (Dooley et al 1989). The role of *H. pylori* in the pathogenesis of gastroduodenal disease has been convincingly established in that the development of acute gastritis occured in two volunteers who ingested *H. pylori* thus satisfying one of Koch's postulates (Marshall et al 1985, Morris et al 1987).

25 This infection has been suggested to play a role in the etiology of gastric cancer (Dunn 1993), in that recent retrospective studies demonstrate a consistently strong association between *H. pylori* and cancer. This relationship refers to adenocarcinomas of body, fundus and antrum but not to cancers of the cardia or gastroesophageal junction.

30 Regarding other gastrointestinal diseases, it appears that, although duodenal ulcer is multifactorial, *H. pylori* is the dominant factor for its pathogenesis. Eradication of *H. pylori* may cure the disease. However, no therapy is available to achieve eradication in all infected individuals.

Some people claim that *H. pylori* may play a role in non ulcer dyspepsia in a proportion of people. Data supporting the above aspect are based on the mucosal changes due to infection, the higher prevelance of *H. pylori* in nonulcer dyspepsia and the beneficial result of eradiation in a substantial number of clinical studies.

Pathogenicity of H. pylori

The success of *H. pylori* as a gastric pathogen is dependent on the following virulence factors and pathogenic mechanisms (Solnick and Tompkins 1993). Various virulence factors such as spiral shape and motility, adaptive enzymes and proteins, and ability to adhere to gastric mucosal cells and mucus allow *H. pylori* to survive in the hostile environment of the gastric lumen. The interaction of *H. pylori* with the gastric mucosa consequences the development of pathogenic mechanisms which lead directly to disruption of the gastric mucosal barrier, including toxins and mediators of inflammation, or contribute to gastric acid-peptic activity. A variety of factors implicated either directly or indirectly in the pathogenesis of *H. pylori* infection are described below.

The spiral shape of the organism and its motility in the mucus, due the presence of unipolar flagella, are essential for colonization of the gastric mucosa. Active motility presumably promotes rapid passage of *H. pylori* through the acidic milieu of the gastric lumen and its prenetration of the gastric mucus layer prior to reaching the neutral environment immediately overlying the gastric epithelium.

Urease hydrolyses urea into ammonia and water. Freshly isolated *H. pylori* stains typically demonstrate high levels of urease activity. Urease appears to play an essential role in infection and development of gastritis induced by *H. pylori*.

The microorganism may be protected from the deleterious effects of gastric acid after colonization has occurred by attaching to mucosal cells and occupying the mucus layer immediatly overlying mucosal cells in an environment in which the pH is close to neutral. Urease activity (NH_3) may also provide a source of nitrogen for *H. pylori*.

Besides its role as a maintenance factor, urease activity may have direct toxic effects on gastric cells and mucus in vivo. A significant correlation

between severity of gastric inflammation and concentration of gastric juice ammonia in *H. pylori*-positive patients with chronic renal failure has been observed (Triebling et al 1991). Accumulation of ammonia produced by hydrolysis of urea may disturb the ionic integrity of gastric mucus and may allow back-diffusion of H^+ toward the gastric mucosa, resulting in tissue injury.

Catalase is an enzyme that protects bacteria against the toxic effects of reactive oxygen metabolites formed in neutrophils from hydrogen peroxide (H_2O_2) as a result of the well-characterized oxidative burst. Catalase hydrolyses H_2O_2 into H_2O and O_2 and thus inhibits formation of reactive oxygen metabolites that kill bacteria through lipid peroxidation and protein denaturation. *H. pylori* catalase is a cytoplasmic enzyme that shares a variety of properties with oher typical bacterial catalases. In vitro, exogenous catalase protects *H. pylori* by preventing the formation of toxic peroxidation products from long-chain saturated fatty acids (e.g., arachidonic acid) (Hazell et al 1991).

Protein inhibitor of gastric acid secretion. Many *Helicobacter* spp inhibit acid secretion from isolated parietal cells (Vargas et al 1991). The inhibitor activity in *H. pylori* is partially heat labile and is inhibited by pretreatment with pronase. The inhibitor is not directly toxic to gastric epithelial cells. Specific inhibition of gastric acid secretion may facilitate acute *H. pylori* infection, especially of parietal cells, and may help explain the transient hypochlorhydria observed in individuals recently infected with *H. pylori*.

Adherence. In vivo, *H. pylori* is associated primarily with gastric mucus-secreting cells, including foci of gastric metaplasia. By electron microscopy, adherence of *H. pylori* to human gastric mucus cells in some cases involves juxtaposition of bacterial and mucosal cell membranes forming "attachment pedestals similar to those observed with adherent enterotoxigenic. *E.coli*. At such sites there is effacement of microvilli and disruption of cytoskeletal elements. In experimentally infected animals, *H. pylori* is found only in the stomach and not in other regions of the gastrointestinal tract. Taken together, these observations suggest that *H. pylori* associates specifically with gastric mucus-secreting cells.

Specificity of bacterial adherence implies interaction between bacterial adhesins and mucosal cell receptors and in that respect, a variety of putative *H. pylori* adhesins have been identified.

A number of putative cellular receptors have been identified, primarily by assessing adherence of *H. pylori* to immobilized lipids including phosphatidylethanolamine, identified previously as a unique glycerolipid present in human gastric mucosal cells and erythrocytes, asialo-ganglioside GM_1, and asialo-GM_2. Receptors with intermediate affinity include ganglioside GM_3 and paragloboside. Conflicting results regarding the role of lactosylceramide as a receptor for *H. pylori* have been reported *H. pylori* also adheres to type IV collagen, laminin, and human mucus. In vitro, *H. pylori* adheres to a large number of both gastric and nongastric cell types. This apparent nonspecificity of binding may be due in part to surface characteristics of the bacterium. There is disagreement regarding the relative hydrophobic or hydrophilic surface nature of *H. pylori*. Thus, although *H. pylori* may bind specifically to gastric mucus-secreting cells, the observed association between *H. pylori* and mucous cells in vivo may be due in part to preferential growth of *H. pylori* in gastric-type mucus.

Cytotoxic effect of broth culture supernatant from 50% to 60% of *H. pylori* isolates has been documented. This effect is induced via a toxin producing nonlethal vacuolization (Cover et al 1990). Vacuolizing activity is heat labile and protease sensitive, suggesting that a protein is involved. Antigenic 128- and 82-kd proteins are present in *H. pylori* broth culture supernatants with vacuolizing activity, either or both of which may represent the cytotoxin.

In naturally infected humans, vacuolizing cytotoxin may be an important virulence factor. Cytotoxic activity is more prevalent in isolates of *H. pylori* from individuals with peptic ulcer disease than in those with gastritis only.

Mucinase. The mucin gel represents the "roof" that protects the underlying submucosal tissue from gastric acid. Chronic *H. pylori* infection is associated with depletion of the mucus layer overlying gastric cells. This depletion of mucus may be due both to inhibition of mucus secretion and to degradation of mucus after it is secreted. Mucinase-associated degradation of gastric mucus would likely disrupt the normal barrier function, facilitating

back-diffusion of H^+ ions and leading to injury of gastric epithelial cells. Reduction of gastric mucus viscosity might also facilitate penetration of *H. pylori* toward the gastric mucosa and release essential nutrients for *H. pylori* survival and growth. In vitro, *H. pylori* secretes a protease capable of degrading porcine gastric mucus. Preincubation with *H. pylori* filtrate leads to loss of the viscoelastic and permselective qualities of gastric mucus. However, mucinase activity has not been detected in all infiltrates of *H. pylori*.

Lipopolysaccharide of *H. pylori* appears to inhibit laminin binding with its receptors epithelial surface. Inhibition of binding laminin with its specific receptor may contribute to the loss of gastric mucosal integrity in *H. pylori* infection.

Lipase and Phospholipase A. H. pylori filtrates exhibit lipase and phospholipase A (PLA) activity. Both enzymes appear to contribute to the degradation of gastric mucus observed in vitro in the presence of *H. pylori* filtrates (Slomiany et al 1989). Mucosal lipids and phospholipids play important roles in maintenance of the viscosity of gastric mucus, prevention of back-diffusion of H^+ ions and maintenance of the hydrophobic lining of the stomach; however, lysophospholipids are deleterious to the integrity of the protective mucus layer (Goggin et al 1991). Thus, *H. pylori* induced formation of lysophospholipids may seriously impair the protective function of the gastric mucus gel. Furthermore, PLA-induced degradation of membrane phospholipids results in formation or arachidonic acid, which can be converted into leukotrienes, prostaglandins, or thromboxanes (Lewis et al 1990) These compounds are known to be chemotactic and can alter cell membrane permeability, enhancing the inflammation process.

Hemolysins produced may be cytotoxic and can mediate inflammation of the gastric mucosa.

Inflammation of the gastric mucosa is thought to decrease the integrity of the gastroduodenal mucosal barrier. This is considered to be mediated through several activities of *H. pylori* such as *invasivity, neutrophil activation, activation of macrophages and monocytes, leukotriene B4, leukocyte migration inhibition, phospholipase A, platelet activating factor (Paf), heat*

shock protein immune stimulation, histamine releasing and eosinophil infiltration and degranulation.

Invasivity. In general, most *H. pylori* bacteria observed in gastric biopsies are closely associated with the surface mucous cells or are present in the gastric mucus; however there are several reports that a small number of *H. pylori* invade the lamina propria in both immunocompetent and immunodeficient hosts (Wyle 1990). This property might have an important consequence in that invasive H. pylori may be able to resist the effects of chemotherapeutic agents, leading to difficulty in eradication.

Neutrophil Activation. Cell-free supernatants from broth culture of *H. pylori* stimulate a significant oxidative burst in neutrophils (Mooney et al 1991).

Activation of Monocytes and Macrophages. Soluble *H. pylori* surface proteins, LPS, or whole cells, can induce expression of the monocyte surface antigen HLA-DR and interleukin-2 receptor, synthesis of the inflammatory cytokines interleukin-1 and tumor necrosis factor and secretion or the reactive oxygen metabolite superoxide anion (Mai et al 1991). In fact, *H. pylori*-infected individuals release significantly greater amounts of tumor necrosis factor-alpha into culture supernatants than do gastric biopsies from uninfected individuals (Mai et al 1991). In vivo, mucosal resorption of secreted *H. pylori* proteins may activate macrophages residing in the lamina propria or mucosal monocytes by mechanisms similar to those described above (Mai et al 1991).

Leukotriene B. Leukotrienes, products of the metabolism of arachidonic acid, are chemotactic and cytotoxic in the gastric mucosa. Levels of leukotriene B_4 (LTB_4) are significantly higher in gastric mucosa infected with *H. pylori* than in uninfected mucosa (Fukuda et al 1990). In addition, LTB_4 levels are significantly higher in gastric mucosa with acute inflammation than in non-inflamed mucosa. These substances are likely to promote additional inflammation.

Leukocyte Migration Inhibition. The migration of leukocytes from *H. pylori*-positive individuals is significantly inhibited in the presence of *H. pylori* antigen when compared with migration in the absence of antigen (Fixa et al

vasoactive mediators from mast cells and facilitate recruitment of additional inflammatory cells because of release of chemotactic mast cell factors (Aceti et al 1991).

Gastrin Hypothesis. The association between gastrin levels and *H. pylori* infection is currently the most thoroughly studied feature relating to pathogenesis in vivo. Prolonged hypergastrinemia associated with *H. pylori* infection may contribute to increased parietal cell mass and chronically increased secretion of gastric acid. Gastrin is a peptide secreted by antral G cells that stimulates parietal cells to secrete acid and, to a lesser extent, chief cells to secrete pepsin. Gastrin secretion is at least as important as is vagal stimulation in control of gastric secretion (Wolfe et al 1988). Individuals with duodenal ulcer and antral *H. pylori* infection had significantly higher basal and meal-stimulated plasma gastrin concentrations and higher peak, but not basal, acid output than individuals with duodenal ulcer who are not infected with *H. pylori*. Eradication of *H. pylori* decreased meal-stimulated gastrin levels, but not basal gastrin, basal acid output, or peak acid output levels. Accordingly it has been proposed that *H. pylori* in the gastric antrum increases antral gastrin release.

Pepsin is an active proteolytic enzyme formed from pepsinogen in the presence of gastric acid and previously formed pepsin. Increasing luminal pepsin activity resulting from elevated gastrin concentrations could degrade the gastric mucus layer, which in turn might enable other aggressive factors, including gastric acid, to injure the underlying epithelium leading to ulcer formation.

PRIOR ART

Principles of antimicrobial therapy

In vitro, *H. pylori* is susceptible to a wide variety of antimicrobial agents, including penicillins, macrolides, nitroimidazoles, quinolones, tetracyclines, and some but not all cephalosporines (McKinlay 1992). *H. pylori* organisms also are susceptible to bismuth-containing compounds, with minimal inhibitory concentrations well below the levels that are clinically achievable in the stomach after oral dosing. Althought resistant to H_2 blockers such as cimetidine and ranitidine, *H. pylori* organisms are susceptible to proton-pump inhibitors such as omeprazole and lansoprazole.

1990). This decreased chemotaxis may contribute to *H. pylori*-associated inflammation by preventing leukocyte emigration from gastric tissue.

Platelet Activating Factor (Paf). Paf-acether, first described as platelet activating factor, is a potent inflammatory mediator produced by both prokaryotic and eukaryotic cells. Paf can produce severe pathologic changes, including gastric ulceration. Paf has been detected in *H. pylori* grown on blood agar plates and under certain conditions when grown in broth. Paf precursors, but not Paf itself, have been detected in gastric biopsy material from *H. pylori*-infected individuals. It is assumed that *H. pylori* add to local production of Paf in the gastric mucosa, promoting mucosal injury.

Autoimmune Phenomena. *H. pylori* stimulates formation of antibodies, evidently produced by heat shock proteins, that cross-react with human antral gastric antigens (Engstrand et al 1991). In addition, there is induced expression of major histocompatibility class II antigens on gastric cells in individuals with *H. pylori*-associated gastritis (Scheynius et al 1991) and an increasing number of μo T cells within the epithelium. These observations suggest that μo T cells play a role in host defense against *H. pylori* infection and that *H. pylori* may trigger an autoimmune response to heat shock (stress) proteins expressed by gastric epithelial cells.

Eosinophil Infiltration dans Degranulation. Infiltration of eosinophils in *H. pylori*-associed antral gastritis is greater as compared to normal controls. Eosinophils may accumulate in the lamina propria because of a bacterial chemostatic factor. IgA secreted locally by plasma cells may stimulate eosinophil degranulation, thus releasing cationic proteins, including major basic protein, which damage the mucosal barrier. The significance of the above process is questionable as eosinophil degranulation did not appear to be greater at or near sites of *H. pylori* infection, nor were significant deposits of major basic protein noted in the margins of ulcers.

Specific Basophil-Bound and Serum IgE. An immunologic response involving IgE has been associated with *H. pylori*-infected individuals but rarely in people uninfected with *H. pylori*. *H. pylori* but not other bacteria, induced histamine release from basophils of infected individuals and adsorbed *H. pylori*-specific serum antibody. On the basis of these observations, *H. pylori* antigen and mast cell-bound IgE in combination could promot release of

From numerous clinical trials the following principles have emerged.

Antimicrobial agents that appear excellent in vitro, may have no efficacy at all in vivo. Erythromycin is an example of such an agent. Antimicrobial failure may be due either to inactivity of the agent at an acid pH or to its inability to achieve adequate concentrations within the gastric mucus.

H. pylori may become resistant to certain agents but not to others. Primary or acquired resistance to nitroimidazoles, quinolones, rifampicin, and macrolides has been reported. In contrast, primary resistance to bismuth salts, penicillins or tetracyclines, despite their widespread uses has not been reported.

From numerous clinical trials it has become clear that it is relatively easy to suppress *H. pylori* with antimicrobial agents, but it is difficult to permanently eradicate this microorganism. Combination therapy has produced better results than monotherapy. This may result from the inability of single agent to reach all organisms in their varied ecological niches within the gastric mucus and mucosa. Combination therapy, by either antimicrobial synergy, or by achievement of increased tissue bactericidal levels, is better able to eradicate the microorganism.

In order to determine whether eradication has occured, it is necessary to evaluate the patient not during therapy or immediately after its completion but some time later. Many therapies appear to be effective if evaluated while they are being given, because bacterial load may be surpressed below the limits of detection; however, after completion of therapy and regrowths of organisms, infection is detectable again (recurrence). At least 3 months but usually 6 months or more must pass before true eradication can be diagnosed reliably.

Therapeutic regimens

The current "gold standard" of therapy for *H. pylori* infection has been termed "triple therapy". It involves the administration of a bismuth salt, 5-nitroimidazole, and either amoxicillin or tetracycline. The 5-nitroimidazole component has emerged as the most critical factor. When a person is infected with an *H. pylori* strain resistant to 5-nitroimidazoles, triple therapy is usually unsuccessful, with eradication rates of <20%. The presence of a resistant strain is associated with prior treatment with a 5-nitroimidazole.

When a person is infected with an *H. pylori* strain that is susceptible to 5-nitroimidazoles, triple therapy is usually successful, with eradication rates >80%. However, in those patients for whom therapy fails, isolates are metronidazole-resistant post treatment but are not resistant to the other components of the regimen. Resistance of *H. pylori* to metronidazole and to other 5-nitroimidazole drugs has emerged worldwide and now constitutes a major problem in therapy (European Study Group 1992).

An alternative regimen involves omeprazole and amoxicillin. Although clinically effective for treatment of ulcers, the two drugs were associated with eradication rates that were somewhat lower than those obtained with triple therapy.

Whilst the number of agents combined has risen, the duration of treatment has progressively fallen. Certainly four week courses are as effective as eight week, and two weeks of therapy may be as good as four.

On the basis of current information, it appears that a reasonable approach to therapy is to treat all patients with triple therapy initially and to consider susceptibility testing and other therapies only for those who fail to benefit from this regimen.

However, in spite of the relatively large number of the antimicrobial regimens used against *H. pylori* some problems have been encountered in the treatment of *H. pylori* infections. First, relapse of *H. pylori* is common following what appears to be successful treatment (Haas et al 1990). Relapse is presumably due to incomplete eradication of the organism. The second major problem has been the emergence of resistant strains during single-agent treatment. The Development of resistance has been reported during treatment with several quinolones, 5-nitroimidazoles and rifampicin.

THE INVENTION

It is an object of the invention to provide a new method for treating diseases related to Helicobacter pylori infections and H. pylori infection related diseases, including gastritis, chronic gastritis, gastric ulcers, duodenal ulcers, gastric carcinoma and non ulcer dyspepsia.

Another object of the invention is to provide a new treatment for Helicobacter infections and related diseases, including those relating to H. pylori.

Another object is to improve the efficiency of treatment of Helicobacter infection related diseases.

Another object is to provide an efficient drug for treating such infections and diseases, which avoids or reduces the occurence of resistant H. pylori strains, and thus to improve eradication of the infection.

Another object is to use clioquinol to prepare a drug which allows one to achieve the above mentioned goals.

The present invention provides a new method for treating Helicobacter, including *H. pylori*, infection related diseases in humans characterized by administrating to a human an efficient quantity of clioquinol, its equivalents or its derivatives in a pharmaceutically acceptable vehicle.

According to the present invention, *H. pylori* infection related diseases include not only acute infection by *H. pylori*, but also diseases in the etiology of which *H. pylori* plays a role, including acute gastritis, chronic gastritis, gastric ulcers, duodenal ulcers, gastric carcinoma, and non ulcer dyspepsia.

According to a preferred embodiment of the invention, clioquinol or its equivalent or derivative is administered by oral route for intra-gastric delivery. The pharmaceutically acceptable vehicle may thus be an usual solid excipient for oral absorption and gastric delivery, for example under the form of tablet, pill, gelule.

Although the posology and the duration of the treatment according to the invention may vary and be adapted to the patient by the skilled physician, it is prefered that the dayly posology is not over 500 mg. According to one embodiment of the invention clioquinol is administered by 10 to 50 mg, and preferably 20 mg twice a day. The duration of the treatment is preferably at least one week but may be of several weeks or even months.

The active principle of the method according to the invention may be used alone or combined with other active principles useful in the treatment of gastritis, gastric or duodenal ulcers, gastric carcinoma, non ulcer dispepsia, and, generally, Helicobacter infections, including e.g. other quinolone type or non quinolone type antibacterial drugs, as for example metronidazole and

other 5-nitroimidazoles, amoxicillin, tetracyclines, bismuth salts, e.g. bismuth subcitrate, in an effective quantity.

The present invention also provides a new drug for treating Helicobacter, including *H. pylori*, infection related diseases, comprising an effective amount of clioquinol, its equivalents or derivatives, in a pharmaceutically acceptable vehicle, preferably an usual excipient suitable for oral absorption and intra-gastric delivery.

According to a prefered embodiment, the drug according to the invention also comprises an effective amount of at least an antibacterial agent, including quinolone type and non quinolone type antibacterial agent, e.g. metronidazole, amoxicillin, a tetracyclin. A very preferred drug according to the invention contains an effective amount of a bismuth salt, more preferably bismuth subcitrate for example 10 to 50 mg. Preferably he weight ratio clioquinol/bismuth salt is between 0,5 and 2.

The present invention also provides the use of clioquinol, its equivalents or derivatives, for preparing a drug for the treatment of Helicobacter, including *H. pylori*, infection related diseases, wherein an effective amount of clioquinol, its equivalents or derivatives is mixed with a pharmaceutically acceptable vehicle, as a dosage form, preferably for oral route administration and intra-gastric delivery.

Clioquinol is an hydroxy-quinolone derivative. Its formula is 7-iodo-5-chloro-8-quinolinol. Equivalent to clioquinol, according to the present invention, comprise other hydroxy-quinolone halogenated derivatives, e.g. iodoquinol (5,7-diiodo-8-hydroxyquinoline), chiniofon (8-hydroxy-7-iodo-5 quinoline sulphonic acid).

Clioquinol derivatives are, e.g. obtained: by substituting the hydrogen atom of the 8-hydroxyle group by a lower alkyle, having preferably from 1 to 3 carbon atomes, most preferably $-CH_3$; and/or substituting the 7-iode atom by lower alkyl or aryl chain, having or not a sulphur or phosphore or oxygen heteroatom; or by substituting the 7 and 8 positions to form a furannic cycle. The methods of preparing such compounds are disclosed by R. Beugelmans et al., Eur. J. Med. Chem. 23 (1988), 539-546, as well as certain compounds.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

1. In vitro activity of clioquinol against *H. pylori*.

Material and Methods

Microorganisms. Thirty five clinical isolates of *H. pylori* were obtained gastroscopically (antrum biopsies) from patients with active gastritis. None of the patients was receiving antibiotics against the microorganism, before biopsy.

The biopsy specimens were homogenized and inoculated on horse, blood agar plates (7% horse blood) prepared as follows:

Twenty five g of GC-Agar Base (BBL) and 3 g of Bacto Agar (Difco) were dissolved in 1,000 mL of ultrapure water; After autoclaving at 121°C for 20 min, horse blood (80 ml) was added, and the temperature was held at 80°C for 20 min to hemolyze the blood. Subsequently, the temperature was lowered to 56°C and 100 ml of horse serum was added. After cooling the medium to 44 to 46°C, 3.5 ml of IsoVitalex (BBL) was added. Finally, 6 mg of vancomycin, 20 mg of nalidixic acid and 2 mg of amphotericin B, which were dissolved in ultrapure water and filter sterilized, were aseptically added to the ready-to-pour-medium. The final pH of the medium was adjusted to 7.2+0.2 before the medium was poured into petri dishes (Soltesz et al 1992).

All cultures were incubated at 37°C in microaerophilic conditions in jars with disposable hydrogen+carbon dioxide generator envelope-GasPak (BBL), without a catatyst, for up to 6 days.

In all cases replacement of the microaerophilic atmosphere took place afer 24 hrs of incubation, as this procedure appeared to enhance the growth of *H. pylori*. The identity of *H. pylori* was confirmed by colony appearance, Gram stain, and positive (oxidase, catalase and urease) as well as negative (hippurate hydrolysis, nitrate reduction) biochemical tests.

All stains were subcultured at least three times in the same medium and grown at microaerophilic conditions to ensure reliable growth and purity.

The strains were maintained in tryptic soy broth (BBL) supplemented with 25% glycerol and 10% horse serum at -70°C.

Prior to testing, isolates were brought up to temperature of 37°C and maintained on the same medium as above in a microaerophilic environment

brain heart infusion broth) of each isolate prepared from a 72-h culture was spread over the surface of the plate. The inoculum ranged from $1x10^6$ to $1x10^8$CFU. The plates were incubated for 4 days. The frequency of spontaneous resistance can be calculated by dividing the colony count on the antibacterial agent-containing plates by the inoculum (Haas et al 1990).

Post-exposure study. Suppresion of growth after short exposure of a suspension *H. pylori* to various concentrations of clioquinicol has been studied.

Bacterial suspension of at least 10^6 FU prepared as previously in brain heart infusion broth and or in PBS supplemented with gelatin added at a final concentration of 1% (w/v) were used. To that bacterial suspension, clioquinol was added at a final concentration of 10 µg/ml. This assay mixture was incubated for 10, 20 and 30 minutes in a microaerohilic atmosphere as described previously. At the end of the exposure time, the suspensiion was centrifuged and the precipitate was resuspended in a drug-free medium (brain heart infusion broth and or PBS with gelation 1%) of such a volume to have finally a suspension of 10^4-10^5 CFU/ml. Appropriate volume of the latter suspension were then plated in drug-free plates as previously prepared. These plates were incubated for up to 4 days in a microaerophilic atmosphere.

Results

The results obtained with clioquinol, bismuth subcitrate and their combinaison against 35 clinical isolates are presented in three tables.

Table 1 shows that the majority (21/35) of the stains of *H. pylori* were inhibited by clioquinol added at a concentration of 10µg/ml. All stains were inhibited at a concentration of clioquinol equal or more than 20 µg/ml.

The testing of the antibacterial activity of bismuth subcitrate showed (table 2) that a substantial proportion (16/35) of *H. pylori* strains were inhibited at a concentration of 10 µg/ml. For inhibition of all strains, a concentration of 20 µg/ml was required.

The activity of the combination of clioquinol with bismuth subcitrate proved more efficient from each drug alone in that an additive effect was observed as clearly shown in table 3. In fact, 21 out of 35 tested strains were inhibited by a concentration of 5 µg/ml of both drugs, whereas all

for 48 h, at which time they demonstrated the characteristics colony morphology and an immediate strong reaction in the oxidase test.

Antibacterial agents. Reference standards for the two drugs were provided by the manufacturers. The drugs tested clioniquinol, an hydroxyquinolone derivative (7-iodo-5-chloro-8-quinolinol, Snow Brand America Inc.) and bismuth subcitrate (Davos Chemicals, Fort Lac, NJ).

Determination of Minimum Inhibitory Concentrations (MICs). MICs were determined by a routine agar dilution technique (1) by using Mueller-Hinton agar (BBL) supplemented with 8% sheep hemolysed blood. Clioquinol's solutions were prepared in dimethylsulfoxide (DMSO), whereas bismuth subctrate was dispersed in water. Serial dilutions of all tested study drugs were prepared with steril water. All antibacterial agents were added to the medium to final concentrations ranging from 1.25 to 160 μg/ml. Drug-containing medium was prepared within 24 h of use and stored overnight at 4°C if necessary.

Isolates were grown for 72 h on blood agar and then suspended in brain heart infusion broth to provide a turbidity approximating an 1.0 McFarland standard. A 1-μl inoculum was applied by using a multipoint inoculator (MIC-2000 Inoculator; Dynatech Laboratories, Inc, Alexandria, Va). The inoculum ranged from $0.36X10^5$ to $5.5X10^7$ CFU. The bacterial inoculum was confirmed by colony counts. Isolates of *Escherichia coli*: ATCC 25922 and a clinical isolate were included on each plate to serve as control organisms.

The plates were incubated for 72 h at 37°C in a humid, microaerophilic environment (CampyPouch System; BBL). The MIC was defined as the lowest concentration (micrograms per mililiter of agar) that inhibited visible growth, disregarding a haze of barely visible growth. MIC testing was performed in duplicate.

MIC interpretive standards for *H. pylori* have not been established. The MIC could be interpreted as a susceptible or resistant result according to concentrations achieved in gastric fluid in a full stomach upon dosage two times daily with 20 mgr of clioquinol and 20 mgr of bismuth subcitrate.

Frequency of spontaneous resistance. Duplicate plates containing the media and antibacterial agents described above were preparared at four and eight times the MIC for each original *H. pylori* isolate. A suspension (0.1 ml

Table 1. Activity of clioquinol against 35 clinical isolates of *H.pylori*

	Growth at defined concentrations of the drug (µg/ml)				
Strain no	1.25	2.5	5	10	20
1	+	+	+	+	-
2	+	+	+	-	-
3	+	+	+	-	-
4	+	+	+	-	-
5	+	+	+	-	-
6	+	+	+	+	-
7	+	+	+	-	-
8	+	+	+	-	-
9	+	+	+	-	-
10	+	+	+	+	-
11	+	+	+	-	-
12	+	+	+	-	-
13	+	+	+	-	-
14	+	+	+	-	-
15	+	+	+	+	-
16	+	+	+	+	-
17	+	+	+	+	-
18	+	+	+	+	-
19	+	+	+	-	-
20	+	+	+	-	-
21	+	+	+	-	-
22	+	+	+	+	-
23	+	+	+	+	-
24	+	+	+	-	-
25	+	+	+	-	-
26	+	+	+	+	-
27	+	+	+	+	-
28	+	+	+	+	-
29	+	+	+	+	-
30	+	+	+	+	-
31	+	+	+	+	-
32	+	+	+	+	-
33	+	+	+	-	-
34	+	+	+	-	-
35	+	+	+	-	-

strains were susceptible to a concentration of 10 or 20 μg/ml of the drug added separately.

Clioquinol does not seem to select resistant variants in that with four strains studied the frequency of spontaneous emergence of resistance was less than $1X10^{-8}$ even at concentration of the drug at 4 times the MIC. The level of detection for these experiments was limited by the size of the inoculum.

Exposure of four strains to clioquinol added at a concentration of 10 μg/ml for at least 10 minutes, resulted in the complete suppression of the growth.

The same method is usefull to detect the in vitro activity of compounds equivalent to clioquinol and to clioquinol derivatives.

2. Examples of preparation of drugs.

- Example 1.

Clioquinol (US patent 641,491) powder is mixed with an usual excipient for gastric release and tablets are prepared with a unit dose of 50 mg clioquinol.

These tablets are administered twice a day.

Clioquinol has a great acid stability and a long half-life. With this galenic form adapted to remain in the stomach it achieves a high concentration in the gastric mucosa.

- Example 2.

Clioquinol powder and bismuth subcitrate powder are mixed with the excipient of example 1 and the mixture treated in a conventional way to form tablets. The unit dose of the tablet is 20 mg of chloroquinol and 20 mg bismuth subcitrate.

The tablets are orally administered twice a day during one week, or two weeks, or four weeks.

Table 2. Activity of colloidal bismuth subcitrate against 35 clinical isolates of *H.pylori*

	Growth at defined concentrations of the drug (µg/ml)				
Strain no	1.25	2.5	5	10	20
1	+	+	+	-	-
2	+	+	+	-	-
3	+	+	+	-	-
4	+	+	+	-	-
5	+	+	+	-	-
6	+	+	+	-	-
7	+	+	+	-	-
8	+	+	+	-	-
9	+	+	+	-	-
10	+	+	-	-	-
11	+	+	-	-	-
12	+	+	+	-	-
13	+	+	+	-	-
14	+	+	+	-	-
15	+	+	+	-	-
16	+	+	+	-	-
17	+	+	+	-	-
18	+	+	+	-	-
19	+	+	+	-	-
20	+	+	+	-	-
21	+	+	+	-	-
22	+	+	+	-	-
23	+	+	+	-	-
24	+	+	+	-	-
25	+	+	+	-	-
26	+	+	+	-	-
27	+	+	+	-	-
28	+	+	+	-	-
29	+	+	+	-	-
30	+	+	+	-	-
31	+	+	+	-	-
32	+	+	+	-	-
33	+	+	+	-	-
34	+	+	+	-	-
35	+	+	-	-	-

Table 3. Activity of clioquinol as combined with colloidal bismuth subcitrate against 35 clinical isolates of *H.pylori*

	Growth at defined concentrations of the drug (µg/ml)			
Strain no	1.25	2.5	5	10
1	+	+	+	-
2	+	+	-	-
3	+	+	-	-
4	+	+	-	-
5	+	+	-	-
6	+	+	-	-
7	+	+	+	-
8	+	+	+	-
9	+	+	+	-
10	+	+	+	-
11	+	+	-	-
12	+	+	-	-
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19	+	+	-	-
20	+	+	-	-
21	+	+	-	-
22	+	+	+	-
23	+	+	+	-
24	+	+	+	-
25	+	+	+	-
26	+	+	+	-
27	+	+	+	-
28	+	+	+	-
29	+	+	+	-
30	+	+	+	-
31	+	+	+	-
32	+	+	+	-
33	+	+	+	-
34	+	+	+	-
35	+	+	+	-

References

Aceti A, Celestino D, Caferro M, et al (1991). Basophil-bound and serum immunoglobulin E directed against *Helicobacter pylori* with chronic gastritis. Gastroenterology 101:131.

Cave TR, Cave DR (1991). *Helicobacter pylori* stimulates pepsin secretion and serum gastrin levels in gastric glands. Scand. J. Gastroenterol. Suppl. 181:9.

Cover TL, Dooley CP, Blaser MJ (1990). Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. Infect. Immun. 58:603.

Dooley CP, Fitzgibbons PL, Cohen H, Appleman MD, Perez-Perez GI, Baser MJ (1989). Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. N. Engl. J. Med. 321:1562-1566.

Dunn BE (1993). Pathogenic mechanisms of *Helicobacter pylori*. Gastroenterology Clinics of North America, 22(1):43.

Engstrand L, Scheymus A, Famson C (1991). An increased number of gamma acid and gastric epithelial cell expression of the groEl stress-protein homologue in *Helicobacter pylori*-associated chronic gastritis of the antrum. Am. J. Gastroenterol. 86:976.

European Study Group of antibiotic susceptibility of *Helicobacter pylori* 1992. Results of a multicenter European survey in 1991 of metronodazole resistance in *Helicobacter pylori*. Eur. J. Clin. Microbiol. Infect. Dis . 11:777-781.

Figura N, Gugliemetti P, Rossolini A, et al (1989). Cytotoxin production by *Campylobacter pylori* strains isolated from

patients with peptic ulcers and from patients with chronic gastritis only. J. Clin. Microbiol. 27:225.

Fixa B, Komarkova O, Krejsek J, et al (1990). Specific celllular immune response in patients with *Helicobacter pylori* infection. Hepatogastroenterology 37:606.

Fukuda T, Kimura S, Arakawa T, et al (1990). Possible role of leukotrienes in gastritis associated with *Campylobacter pylori*. J. Clin. Gastroenterol. 12(Suppl.1):S131.

Goggin PM, Northfield TC, Spychal RT (1991). Factors affecting gastric mucosal hydrophobicity in man. Scand J Gastroenterol Suppl 181:65.

Haas CE, Nix DE, Schentag JJ (1990). In vitro selection of resistant *Helicobacter pylori*. Antim. Agents and Chem. 9:1637-1641.

Hazell SL, Evans JD Jr, Graham DY (1991). *Helicobacter pylori* catalase. J. Gen. Microbiol. 137:57.

Lewis RA, AUsten KF, Soberman RJ (1990). Leukotrienes and other products of the 5-lipoxygenase pathway. N. Engl. J. Med. 323:645.

Mai UEH, Perez-Perez GI, Wahl LM et al (1991). Soluble surface proteins from *Helicobacter pylori* activate monocytes/macrophages by lipopolysaccharide-independent mechanism. J. Clin. Invest. 87:894.

Marshall BJ, Armstrong JA, McGechie DB, Glandy RJ (1985). Attempt to fulfill Koch's postulates for pyloric *Campylobacter*. Med. J. Aust. 142:436-439.

Marshall BJ, Warren JR (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration [letter]. Lancet i:1311-1314.

McKinlay A (1992). Antibiotics in the treatment of peptic ulcer disease. J. ANtimicrob. Chem. 29:91-96.

Mooney C, Keenan J, Munster D, et al (1991). Neutrophil activation by *Helicobacter pylori.* Gut 32:853.

Morris A, Nicholson G (1987). Ingestion of *Campylogacter pyloris* causes gastritis and raised fasting pH. Am. J. Gastroenterol. 87:192-198.

Scheynius A, Engsstrand L (1991). Gastric epithelial cells in *Helicobacter pylori*-associated gastritis express HLA-DR but nor ICAM-1. Scand. J. Immunol. 33:237.

Slomiany BL, Nishikawa H, Plotrowski J, et al (1989). Lipolytic activity of *Campylobacter pylori.* Effect of sofalcone. Digestion 43:33.

Solnick J, Tompkins L (1993). *Helicobacter pylori* and gastroduodenal disease: pathogenesis and host-parasite interaction. Infectious Agents and Disease 1:294-309.

Soltesz V, Zeeberg B, Wadstrom T (1992). Optimal survival of *Helicobacter pylori* under various transport conditions. J. Clin. Microbiol. 1992, 30:1453-1456.

Triebling AT, Korsten MA, Dlugosz JW, et al (1991). Severity of *Helicobacter*-induced gastric injury correlates with gastric juice ammonia. Dig. Dis. Sci 36:1089, 1991.

Vargas M, Lee A, Fox JG, et al (1991). Inhibition of acid secretion from parietal cells by non-human-infecting *Helicobacter* species: A factor in colonization of gastric mucosa? Infect. Immun. 59:3694.

Warren JR, Marshall B (1983). Unidentified curved bacillin on gastric epithelium in active chronic gastritis [letter]. Lancet i:1273-1275.

Wolfe MM, Soll AH (1988). The physiology of gastric acid secretion. N. Engl. J. Med. 319:1707.

Wyle FA, Tarnawski A, Schulman D, et al (1990). Evidence for gastric mucosal cell invasion by *C.pylori.* An ultrastructural study. J. Clin. Gastroenterol. 12(suppl. 1):S92.

CLAIMS

1. A method for treating Helicobacter, including *H. pylori*, infection related diseases in humans characterized by administrating to a human an efficient quantity of clioquinol, its equivalents or its derivatives in a pharmaceutically acceptable vehicle.

2. A method according to claim 1 for treating chronic gastritis, gastric ulcers, duodenal ulcers, gastric carcinoma, and non ulcer dyspepsia.

3. A method according to claim 1 or 2 wherein clioquinol or its equivalent or derivative is administered by oral route for intra-gastric delivery.

4. A method according to claim 1, 2 or 3, wherein clioquinol is administered by 10 to 50 mg, and preferably 20 mg twice a day.

5. A method according to any one of claims 1 to 4 including administration of at least another quinolone type or non quinolone type antibacterial drugs, as for example metronidazole and other 5-nitroimidazoles, amoxicillin, tetracyclines.

6. A method according to any one of claim 1 to 4 wherein an effective amount of a bismuth salt, preferably bismuth subcitrate, is simultaneously administered.

7. A method according to claim 6 wherein bismuth salt is administered by 10 to 50 mg, preferably 20 mg.

8. A new drug for treating Helicobacter, including *H. pylori*, infection related diseases, comprising an effective amount of clioquinol, its equivalents or derivatives, in a pharmaceutically acceptable vehicle.

9. A drug according to claim 8 comprising an usual excipient suitable for oral absorption and intra-gastric delivery.

10. A drug according to claim 8 or 9 also comprising an effective amount of at least one antibacterial agent, including quinolone type and non quinolone type antibacterial agent, e.g. metronidazole and other 5-nitroimidazoles, amoxicillin, tetracyclines.

11. A drug according to claim 8 or 9 which contains an effective amount of a bismuth salt, preferably bismuth subcitrate.

12. A dosed form of drug according to any of claims 8 to 10 comprising clioquinol, its equivalents or derivatives by an amount of 10 to 50 mg, preferably 20 mg.

13. A dosed form of drug according to any one of claims 8 to 12, comprising a bismuth salt, preferably bismuth subcitrate, by an amount of 10 to 50 mg, preferably 20 mg.

14. The use of clioquinol, its equivalents or derivatives, for preparing a drug for the treatment of Helicobacter, including *H. pylori*, infection related diseases including chronic gastritis, gastric ulcers, duodenal ulcers, gastric carcinoma, and non ulcer dyspepsia, wherein an effective amount of clioquinol, its equivalents or derivatives is mixed with a pharmaceutically acceptable vehicle, as a dosage form, preferably for oral route administration and intra-gastric delivery.

15. The use according to claim 14 for preparing a drug according to any one of claims 8 to 14.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 098 577 (HOECHST AG) 18 January 1984 see claims ---	8-13
X	INDIAN J.PHARM.SCI., vol.48, no.6, 1986 pages 195 - 7 see page 195, left column, line 1 - line 8 ---	8-13
X	PHARMATHERAPEUTICA, vol.4, no.4, 1985 pages 251 - 4 see page 252, line 14 - line 16 --- -/--	8-13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1994	30. 12. 94

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 455 475 (RECKITT AND COLMAN) 6 November 1991 see page 10, line 20 see page 10, line 39 - line 41 see page 10, line 46 - line 55 see page 11; table 3 see page 12, line 25 - line 27	1-14
X	WO,A,92 18111 (SMITHKLINE BEECHAM) 29 October 1992 see page 4, line 24 see claim 2, line 16 see claim 3, line 30 see claim 4, line 5 see claim 8, line 32	1-14
X	REV.PRAT., vol.39, 1989 page 14 see page 1278, chapters 2,3	8-13

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0098577	18-01-84	DE-A-	3225367	12-01-84
		JP-A-	59042315	08-03-84
EP-A-0455475	06-11-91	AU-B-	645555	20-01-94
		AU-A-	7596891	07-11-91
		GB-A,B	2243549	06-11-91
		US-A-	5286492	15-02-94
WO-A-9218111	29-10-92	AU-A-	1430492	17-11-92
		EP-A-	0580627	02-02-94
		JP-T-	6506919	04-08-94